PPARγ activation attenuates glucose intolerance induced by mTOR inhibition with rapamycin in rats

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Festuccia WT, Blanchard P, Belchior T, Chimin P, Paschoal VA, Magdalon J, Hirabara SM, Simões D, St-Pierre P, Carpinelli A, Marette A, Deshaies Y. PPARγ activation attenuates glucose intolerance induced by mTOR inhibition with rapamycin in rats. Am J Physiol Endocrinol Metab 306: E1046–E1054, 2014. First published March 11, 2014; doi:10.1152/ajpendo.00683.2013.—mTOR inhibition with rapamycin induces a diabetes-like syndrome characterized by severe glucose intolerance, hyperinsulinemia, and hypertriglyceridemia, which is due to increased hepatic glucose production as well as reduced skeletal muscle glucose uptake and adipose tissue PPARγ activity. Herein, we tested the hypothesis that pharmacological PPARγ activation attenuates the diabetes-like syndrome associated with chronic mTOR inhibition. Rats treated with the mTOR inhibitor rapamycin (2 mg·kg–1·day–1) for 15 days were evaluated for insulin secretion, glucose, insulin, and pyruvate tolerance, skeletal muscle and adipose tissue glucose uptake, and insulin signaling. Rosiglitazone corrected fasting hyperglycemia, attenuated the glucose and insulin intolerances, and abolished the increase in fasting plasma insulin and C-peptide levels induced by rapamycin. Surprisingly, rosiglitazone markedly increased the plasma insulin and C-peptide responses to refeeding in rapamycin-treated rats. Furthermore, rosiglitazone partially attenuated rapamycin-induced glycogenogenesis, as evidenced by the improved pyruvate tolerance and reduced mRNA levels of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Rosiglitazone also restored insulin’s ability to stimulate glucose uptake and its incorporation into glycogen in skeletal muscle of rapamycin-treated rats, which was associated with normalization of Akt Ser473 phosphorylation. However, the rapamycin-mediated impairments of adipose tissue glucose uptake and incorporation into triacylglycerol were unaffected by rosiglitazone. Our findings indicate that PPARγ activation ameliorates some of the disturbances in glucose homeostasis and insulin action associated with chronic rapamycin treatment by reducing glycogenogenesis and insulin secretion and restoring muscle insulin signaling and glucose uptake.

mechanistic target of rapamycin; rapamycin; peroxisome proliferator-activated receptor-γ; rosiglitazone; insulin secretion; glycogenogenesis; glucose uptake

MECHANISTIC TARGET OF RAPAMYCIN (mTOR) is an ubiquitously expressed serine-threonine kinase that acts as the catalytic core of two multiprotein complexes of different protein compositions and biological functions (22). Indeed, mTOR complex 1 (mTORC1) is a major positive regulator of protein, lipid, and pyrimidine synthesis controlling cell metabolism, size, and proliferation according to the availability of nutrients, growth factors, and cell energy status (5, 33, 45). The mTOR complex 2 (mTORC2), on the other hand, which is activated mainly by growth factors, acts upon Akt/PKB and other proteins modulating cell metabolism and survival (45).

Because of its role as a master regulator of important metabolic processes essential for proper cell function, dysfunction of the mTOR signaling pathway is most frequently associated with disease development. Indeed, chronic mTORC1 overactivation is a major phenotype found in pathological conditions such as tuberous sclerosis complex disease (17), many types of cancer (29), obesity, and type 2 diabetes (18, 40). With regards to the latter, there is compelling evidence indicating that obesity-induced mTORC1 overactivation in liver, skeletal muscle, and fat is a crucial event in the development of obesity associated insulin resistance (18, 37, 41). Indeed, mTORC1 overactivation promotes insulin resistance by activating its downstream target S6K1, which in turn catalyzes the inhibitory phosphorylation of serine residues on insulin receptor substrate-1 (IRS-1), thus impairing IRS-1 function and PI3K activation by insulin (37–39, 42).

Such major involvement of mTORC1 overactivation in chronic diseases has created the need for the development of highly specific mTOR inhibitors aiming to counteract the negative impact of deregulation in this signaling pathway on body homeostasis. Hence, the bacterial macrolide rapamycin and its analogs, which interact with FK506 binding protein of 12 kDa (FKBP12) and allosterically inhibit some but not all mTORC1 functions (36), have been used in the treatment of diseases characterized by aberrant mTOR activation (29) and as immunosuppressants in transplanted patients (3). In fact, rapamycin has met with limited success in clinical practice, which can be attributed to its restricted inhibitory action toward only a few specific mTORC1 functions (36) and to the development of several major metabolic side effects, including dyslipidemia, insulin resistance, and disturbances in glucose homeostasis (1, 7, 11, 15, 16, 27, 30). Mechanistically, whereas rapamycin induces dyslipidemia mainly by reducing adipose tissue lipid uptake and deposition, likely through inhibition of lipoprotein lipase and PPARγ activities (6, 16), it causes hyperglycemia by increasing hepatic glycogenogenesis (16) and by impairing insulin-stimulated glucose uptake in skeletal muscle (9). Surprisingly, prolonged rapamycin treatment induces insulin resistance despite inhibiting mTORC1 and alleviating S6K-mediated negative feedback on IRS-1 function (16, 37, 42) in part due to mTORC2 inhibition and reduced Akt/PKB phosphorylation (21, 43). Noteworthy is that, along...
with Akt phosphorylation, rapamycin treatment strongly reduces the content and activity of peroxisome proliferator-activated receptor (PPARγ) (6, 16, 19), a nuclear receptor involved, through several mechanisms, in the regulation of whole body glucose homeostasis. Indeed, pharmacological PPARγ activation with thiazolidinediones is associated with a substantial improvement in whole body insulin sensitivity that might be attributed to the increase in peripheral glucose uptake and adiponectin levels and to the reduction in lipidemia and inflammation induced by these compounds (2, 13, 14, 31). Thus, we tested herein the hypothesis that not only is PPARγ involved in the disturbances in glucose homeostasis induced by rapamycin but that its pharmacological activation by thiazolidinedione attenuates the diabetes-like syndrome induced by rapamycin therapy.

**MATERIALS AND METHODS**

Animals. Animal handling and treatment were approved by the Animal Care and Handling Committee of Laval University and Institute of Biomedical Sciences, University of São Paulo. Male Sprague-Dawley rats (Charles River Laboratories or Institute of Biomedical Sciences Animal Facility) were housed individually in a room kept at 23 ± 1°C with a 12:12-h light-dark cycle. After an adaptation period, rats were matched by weight and divided into four groups: control, rapamycin, rosiglitazone, and rapamycin plus rosiglitazone (AVANDIA) at a dose of 15 mg·kg⁻¹·day⁻¹. Vehicle (0.1% Me₂SO, 0.2% carboxymethylcellulose) or rapamycin (2 mg·kg⁻¹·day⁻¹) (LC laboratories, Woburn, MA) was injected intraperitoneally (ip) once daily. The rapamycin dose was chosen based on its efficiency to inhibit the mTOR pathway in rodents, with such doses being within the range of those used in human studies (26, 27). Rats were fed a nonpurified powdered rodent diet (Charles River Laboratories) supplemented with the PPARγ ligand rosiglitazone (2 mg·kg⁻¹·day⁻¹) (LC laboratories, Woburn, MA) was injected intraperitoneally (ip) once daily. The rapamycin dose was chosen based on its efficiency to inhibit the mTOR pathway in rodents, with such doses being within the range of those used in human studies (26, 35). Rats were fed a nonpurified powdered rodent diet (Charles River Rodent Diet No. 5075; Charles River Laboratories, Woodstock, ON, Canada) alone (control) or supplemented with the PPARγ ligand rosiglitazone (AVANDIA) at a dose of 15 mg·kg⁻¹·day⁻¹ for 15 days. This dose of rosiglitazone was shown to improve plasma lipid profile and glucose homeostasis in rodents (13, 23). After 15 days of treatment, rats were euthanized by decapitation for tissue and blood harvesting after a 12-h fasting period, followed by 3 h of ad libitum refueling.

**Intraperitoneal glucose, insulin, and pyruvate tolerance tests.** Rats were fasted for 6 h and injected ip with glucose (2 g/kg) or insulin (2.5 IU/kg, human insulin; Eli Lilly, Toronto, ON, Canada). Plasma glucose disappearance rate (Kitt, %/min) in the insulin tolerance test was calculated as the product of the linear regression slope multiplied by 100 and divided by initial time 0 glyceremia. The slope (regression coefficient) of the linear regression was calculated by the least square method of the logarithm of plasma glyceremia plotted against time 0–60 min after insulin injection. For the pyruvate tolerance test, rats were fasted for 12 h and refed for 3 h before being injected with pyruvate (2 g/kg; Sigma-Aldrich, Oakville, ON, Canada). Glyceremia was measured at various times after pyruvate injection (as indicated in figures).

**Plasma determinations.** Plasma adiponectin was measured by ELISA, following the supplier’s recommendations (Alpco Diagnostics, Salem, NH). Plasma triacylglycerol (TAG; Roche Diagnostics, Montreal, QC, Canada) and nonesterified fatty acid (NEFA; Wako Chemicals, Richmond, VA) levels were measured by enzymatic methods according to the manufacturer’s instructions.

**Pancreatic islet isolation and in vitro evaluation of insulin secretion.** Islets were isolated by pancreas digestion with collagenase, as described previously (20), and pooled in Krebs-Henseleit buffer (in mmol/l: 115 NaCl, 24 NaHCO₃, 5 KCl, 1 MgCl₂, and 1 CaCl₂, pH 7.4) supplemented with 0.2% BSA and gassed with 95% O₂–5% CO₂ at 37°C. Groups of five islets were incubated at 37°C for 60 min in 0.5 ml of the same buffer supplemented with glucose at 5.6 or 16.7 mmol/l. Medium and islet insulin content were measured by RIA. Rates of insulin secretion were calculated as secreted insulin per total islet insulin content.

**Skeletal muscle glucose uptake and incorporation into glycogen.** Soleus muscles were isolated, carefully separated into longitudinal muscle strips (30 ± 3 mg), attached to stainless-steel clips to maintain resting tension, and preincubated at 35°C in Krebs-Ringer bicarbonate buffer (in mmol/l: 118 NaCl, 4.8 KCl, 1.25 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, and 25 NaHCO₃, pH 7.4, gassed with 95% O₂–5% CO₂ containing 5.6 mM glucose). After 30 min, muscles were transferred to vials containing Krebs buffer supplemented with 0.3 μCi/ml α-[U-¹⁴C]glucose and 0.2 μCi/ml 2-deoxy-α-[²,⁶-³H]glucose in the presence or absence of insulin (0.7 or 7 mmol/l), gassed for 15 min, and incubated for 1 h. At the end of incubation, muscles were washed and frozen in liquid N₂ for later analysis of 2-deoxy-α-[²,⁶-³H]glucose uptake and α-[U-¹⁴C]glucose incorporation into glycogen, as described previously (8).

**Adipose tissue glucose uptake and incorporation into triacylglycerol.** Glucose uptake and incorporation into adipose tissue TAG in vitro were measured as described previously (13). Briefly, subcutaneous inguinal fat explants (50 mg) were incubated in Krebs-Ringer bicarbonate buffer containing 5.5 mmol/l of either [¹⁴C]glucose (0.5 μCi/tube; Amersham) or 2-deoxy-α-[²,⁶-³H]glucose (0.5 μCi/tube; Amersham) and 2.5% fatty acid-free bovine serum albumin (Sigma, Oakville, ON, Canada), pH 7.4, for 1 h at 37°C in the presence of insulin (400 pmol/l). Reactions were stopped with H₂SO₄, and explants were destined to either lipid extraction or to the determination of 2-deoxy-[¹-²H]glucose 6-phosphate content, as described previously (12). Results are expressed as micromoles per gram of tissue per hour.

**Immunoblotting.** Tissue samples were homogenized, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes, as described previously (16). Densitometric analysis was performed with ImageQuant TL software (GE Healthcare, Little Chalfont, UK).

**RNA extraction and quantitative PCR analysis.** RNA extraction and quantitative PCR analysis were performed as described previously (13). The primers used are listed in Table 1. Data are expressed as the ratio between the expression of the target gene and the housekeeping gene 36B4, the expression of which was not significantly affected by rapamycin and/or rosiglitazone treatment.

**Statistical analysis.** Results are expressed as means ± SE. Multifactorial ANOVA followed by Newman-Keuls’ multiple range test was used for multiple comparisons. *P < 0.05* was taken as the threshold of significance.

**Table 1. Primer sequences for real-time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>PEPCK</td>
<td>TGGGTGTAGGAGAACTGCTGG</td>
<td>TGGGTGTAGGAGAACTGCTGG</td>
</tr>
<tr>
<td>G6Pase</td>
<td>GAGCTGGCTACCTCAGAAGTGC</td>
<td>TCCCTGGTCACTCCAGACAG</td>
</tr>
<tr>
<td>PGCα</td>
<td>TCTCCTTACTATATTGAATGCAAGGCC</td>
<td>TCTCCTTACTATATTGAATGCAAGGCC</td>
</tr>
<tr>
<td>36B4</td>
<td>TAAAGACTGCGGAGACAAAGTGG</td>
<td>TGGTATCCTGCTCCACAG</td>
</tr>
</tbody>
</table>

PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; PGCα, PPARγ coactivator-α.
RESULTS

Rapamycin treatment significantly reduced body weight gain (−87%), an effect that was due to a reduction in both food intake (−21%) and feed efficiency (−84%) (Table 2). These rapamycin effects were well preserved in rats concomitantly treated with the PPARγ ligand rosiglitazone. Despite reducing body weight gain, rapamycin significantly increased NEFA (34%) and cholesterol (37%) and tended to increase TAG levels. Among these rapamycin effects, rosiglitazone completely abolished the increase in NEFA but not that in cholesterol or TAG levels. No effect of rapamycin alone was seen on plasma adiponectin levels. As expected, rosiglitazone significantly increased plasma adiponectin, an effect that was partially attenuated by rapamycin.

Confirming previous studies, rapamycin severely deteriorated glucose homeostasis, as evidenced by the higher basal glucose levels and higher glycemia excursion and area under the curve in response to glucose injection (Fig. 1, A and B).

Along with hyperglycemia, rapamycin-treated rats had higher insulinemia before and throughout the glucose tolerance test (Fig. 1C). In addition to glucose intolerance, rapamycin significantly reduced insulin responsiveness, as evidenced by the smaller reduction in glycemia and impaired rates of plasma glucose disappearance (Kitt; Fig. 1, D and E, respectively) in response to insulin. Rosiglitazone significantly ameliorated the disturbances in glucose homeostasis induced by rapamycin, as evidenced by the complete correction of 6-h fasting hyperglycemia, attenuation of glucose intolerance and 6-h fasting hyperinsulinemia, and improvement in insulin responsiveness (Fig. 1, A–D). This improvement in the glucose tolerance of rapamycin-treated rats induced by rosiglitazone was not associated with significant changes in plasma insulin levels after glucose injection and throughout the glucose tolerance test (Fig. 1C).

To further evaluate the effects of rapamycin on glucose and insulin homeostasis, plasma glucose, insulin, and C-peptide levels were measured in rats either fasted overnight for 12 h or refed for 3 h after a 12-h overnight fast. Rapamycin-treated rats had higher glucose, insulin, and C-peptide levels in both fasted and refed conditions (Fig. 2, A–C). Upon fasting, rosiglitazone completely corrected rapamycin-induced hyperglycemia and hyperinsulinemia and brought C-peptide to levels of control rats. Upon refeeding, rosiglitazone corrected rapamycin-induced hyperglycemia, but surprisingly, it further increased the already high insulin and C-peptide levels found in rapamycin-treated rats. Studies in isolated islets also showed that rapamycin induces higher rates of insulin secretion in response to 5.6 mmol/l glucose, which was completely abolished by rosiglitazone (Fig. 2D). Similar insulin responses were observed in the presence of 16 mmol/l glucose.

To further explore the mechanisms underlying the disturbances in glucose homeostasis induced by rapamycin and its amelioration by rosiglitazone, the effects of these compounds on hepatic glucose production and adipose tissue and skeletal muscle glucose uptake were evaluated. Confirming previous findings (16), rapamycin significantly increased hepatic glucose production, as evidenced by the higher glycemia at 90 and 120 min after pyruvate injection (Fig. 3A), and elevated mRNA levels of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and PPARγ coactivator-α (PGCα) (Fig. 3B). This rapamycin-induced increase in hepatic glucose production was attenuated partially by rosiglitazone, as evidenced by the partial correction of the hyperglycemic response to pyruvate and complete abrogation of the increases in PEPCK and G6Pase mRNA levels induced by rapamycin (Fig. 3, B and C). Conversely, the elevation in liver PGCα mRNA levels induced by rapamycin was not affected by rosiglitazone (Fig. 3D).

In addition to hepatic glucose production, a likely involvement of adipose tissue and skeletal muscle glucose uptake in the improvement of glucose homeostasis induced by rosiglitazone was investigated. As expected, rapamycin significantly reduced insulin-stimulated glucose uptake and incorporation into TAG in inguinal adipose tissue (Fig. 4, A and B). Confirming previous findings, rosiglitazone alone significantly increased insulin-stimulated glucose uptake in controls, but it failed to improve the lower rates of glucose uptake in adipose tissue of rapamycin-treated rats (Fig. 4A). In contrast to glucose uptake, rosiglitazone significantly increased rates of glucose incorporation into TAG in both control and rapamycin-treated rats, an effect that was of lesser magnitude in the latter (Fig. 4B).

Similarly to adipose tissue, rapamycin significantly reduced insulin-stimulated glucose uptake and incorporation into glycogen in skeletal muscle (Fig. 5, A–D). Rosiglitazone, on the other hand, partially restored insulin’s ability to increase glucose uptake and its incorporation into glycogen in skeletal muscle of rapamycin-treated rats (Fig. 5, A–D).

In an attempt to elucidate the mechanisms underlying the changes glucose homeostasis induced by rapamycin and/or

Table 2. Body weight and gain, energy balance determinants, and blood analytes of rats treated with Rapa in combination or not with RSG for 15 days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RSG</th>
<th>Rapa</th>
<th>Rapa + RSG</th>
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<tr>
<td>Initial weight, g</td>
<td>231 ± 2.3a</td>
<td>230 ± 2.4a</td>
<td>230 ± 2.5a</td>
<td>231 ± 2.5a</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>345 ± 3.2a</td>
<td>348 ± 4.2a</td>
<td>244 ± 3.6a</td>
<td>247 ± 4.3a</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>112 ± 2.6a</td>
<td>118 ± 4.3a</td>
<td>13.8 ± 3.6b</td>
<td>15.8 ± 2.9b</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>174 ± 2.6a</td>
<td>184 ± 3.1b</td>
<td>137 ± 3.8b</td>
<td>126 ± 3.6b</td>
</tr>
<tr>
<td>Food efficiency, %</td>
<td>64 ± 1.1a</td>
<td>63.97 ± 2.1a</td>
<td>9.9 ± 2.5b</td>
<td>12.3 ± 2.2b</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.23 ± 0.032a</td>
<td>0.065 ± 0.004b</td>
<td>0.31 ± 0.04a</td>
<td>0.10 ± 0.012a</td>
</tr>
<tr>
<td>TAG, mmol/l</td>
<td>1.28 ± 0.03a</td>
<td>0.57 ± 0.05a</td>
<td>1.83 ± 0.25a</td>
<td>1.44 ± 0.15a</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>1.95 ± 0.14a</td>
<td>2.17 ± 0.05a</td>
<td>2.69 ± 0.16b</td>
<td>2.88 ± 0.18b</td>
</tr>
<tr>
<td>Adiponectin, μg/ml</td>
<td>2.19 ± 0.09b</td>
<td>9.75 ± 1.12a</td>
<td>3.0 ± 0.27a</td>
<td>5.24 ± 0.32a</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6–12 rats. Rapa, rapamycin; RSG, rosiglitazone; NEFA, nonesterified fatty acids; TAG, triacylglycerol. *Calculated as g body weight gain/100 g food ingested. Means not sharing a common letter are significantly different from each other; P < 0.05.
rosiglitazone, a likely involvement of alterations in liver and skeletal muscle insulin signaling was investigated. As depicted in Fig. 6, A, C, D, and F, rapamycin significantly inhibited mTORC1, as evidenced by the marked reduction in liver and skeletal muscle content of phospho (p)-S6 Ser236. Rosiglitazone treatment, on the other hand, did not affect liver or muscle p-S6 content in either control or rapamycin-treated rats. In contrast to p-S6, the content of p-Akt Ser473 was modulated in a tissue-specific manner by rapamycin and rosiglitazone. In the liver, rapamycin did not affect p-Akt content, whereas rosiglitazone induced a significant reduction of similar magnitude in p-Akt in both control and rapamycin-treated rats (Fig. 6B). In contrast to the liver, rapamycin significantly reduced skeletal muscle pAkt content, an effect that was completely abolished by rosiglitazone (Fig. 6E).

**DISCUSSION**

The previous finding that pharmacological mTOR inhibition reduces the content and transcriptional activity of PPARγ (6, 16, 19), which is involved in the regulation of glucose homeostasis, motivated us to test the hypothesis that pharmacological PPARγ activation would ameliorate the diabetes-like syndrome induced by rapamycin. Our main findings indicate that rosiglitazone administration strongly mitigates the diabetes-like syndrome induced by rapamycin, as evidenced by the improvement in glucose and insulin tolerance, complete cor-
rection of both fasting hyperglycemia and hyperinsulinemia, reduction in hepatic gluconeogenesis, and the restoration of insulin’s ability to increase skeletal muscle p-Akt Ser473 content and glucose uptake. Altogether, these findings indicate that pharmacological PPARγ activation may be an efficient strategy to minimize the disturbances in glucose homeostasis and insulin sensitivity typically seen upon rapamycin therapy.

Confirming previous findings, rosiglitazone did not affect the reductions induced by rapamycin in body weight gain, food intake, or food efficiency, the latter being a consequence of increased energy expenditure (6). Although the underlying mechanisms of rapamycin actions on energy balance are unknown, such effects do not seem to be due to changes in food absorption, as estimated by fecal energy content or changes in

Fig. 2. A–C: plasma glucose (A), insulin (B), and C-peptide levels (C) in rats treated with Rapa or RSG for 15 days and fasted or refed for 3 or 12 h after 12-h fasting (n = 7 for each group). D: glucose-stimulated insulin secretion by isolated islets of rats treated with Rapa or RSG for 15 days (n = 4 for each group). Means not sharing a common letter are significantly different from each other, P < 0.05.

Fig. 3. Pyruvate tolerance test and liver mRNA levels of gluconeogenic enzymes in rats treated with Rapa or RSG for 15 days; n = 6–8 for each group. *P < 0.05 vs. control and RSG-treated rats; #P < 0.05 vs. all groups. Means not sharing a common letter are significantly different from each other; P < 0.05.
nonshivering thermogenesis and as evaluated by brown fat uncoupling protein 1 mRNA levels (data not shown). Similarly to energy balance, rosiglitazone was also ineffective in ameliorating the dyslipidemia induced by rapamycin. Despite significantly reducing circulating NEFA levels, rosiglitazone not only did not affect hypercholesterolemia but also lost its hypotriglyceridemic action in rapamycin-treated rats. These latter findings corroborate those of a previous study showing that pharmacological mTOR inhibition impairs the ability of rosiglitazone to induce lipoprotein lipase activity, lipid clearance, and deposition in adipose tissue (6).

In contrast to energy balance and dyslipidemia, however, PPARγ activation was very effective in ameliorating the abnormalities of glucose homeostasis induced by rapamycin. Indeed, rosiglitazone markedly attenuated both glucose and insulin intolerance and completely corrected the fasting hyperglycemia and hyperinsulinemia induced by rapamycin. Surprisingly, such correction of rapamycin-induced hyperinsulinemia by rosiglitazone depended upon the nutritional status. In contrast to the reduction seen at fasting, rosiglitazone increased further upon refeeding the already high plasma insulin levels of rapamycin-treated rats. In line with an implication of pancreatic β-cell insulin secretion rather than clearance in this phenotype, changes in circulating C-peptide levels closely followed those in plasma insulin induced by rapamycin and rosiglitazone. Furthermore, in agreement with plasma hormone levels, isolated islets from rats treated with rapamycin had elevated in vitro rates of insulin secretion, an effect that was

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)
exacerbate in vitro insulin secretion, an effect that was counteracted by rictor deletion and reduction in mTORC2 activity (24). Noteworthy is that, in contrast to these findings, some studies have found either no effect (10) or impairment of insulin secretion upon treatment with rapamycin and analogs (4, 44). Different animal species or cell lines, rapamycin doses, and treatment duration might account for such discrepant results. Notwithstanding such discrepancies, our findings indicate that in isolated islets, PPARγ activation, through unknown mechanisms, effectively attenuates the insulin hypersecretion induced by rapamycin, which itself seems to be due to mTORC1 rather than mTORC2 inhibition by this macrolide.

To gain further insight into the mechanisms by which pharmacological PPARγ activation attenuates the disturbances of glucose homeostasis induced by rapamycin, rosiglitazone effects on the major processes determining glycemia in non-absorptive periods, namely hepatic glucose production through gluconeogenesis and peripheral glucose uptake, were evaluated. Despite the complete abrogation of the increase in the mRNA levels of the key gluconeogenic enzymes PEPCK and G6Pase, rosiglitazone only partially attenuated the rapamycin-induced elevation of gluconeogenesis, as estimated by a pyruvate tolerance test. These findings strongly indicate the involvement of mechanisms other than changes in gene expression as mediators of the gluconeogenesis upregulation induced by rapamycin. Interestingly, the reduction in gluconeogenesis and PEPCK and G6Pase gene expression induced by rosiglitazone occurred without any significant change in the mRNA levels of PGCα, a nuclear receptor coregulator known to act as a positive regulator of gluconeogenesis (32). Despite these findings, an implication of PGCα in the changes in gluconeogenesis and PEPCK and G6Pase expression should not be excluded, considering the recent findings that PGCα phosphorylation by the mTORC1 downstream substrate S6K is a more important event in the regulation of gluconeogenesis than changes in PGCα protein content (25). Previous studies have suggested that rapamycin upregulates gluconeogenesis by reducing hepatic mTORC2 activity and Akt activation (21). However, we were unable either here or in a previous study (16) to confirm this hypothesis, as evidenced by the absence of change in the hepatic content of p-Akt Ser473 upon rapamycin treatment. Surprisingly, rosiglitazone alone or in combination with rapamycin significantly reduced liver p-Akt Ser473 content, further indicating that the attenuation of rapamycin-induced gluconeogenesis by rosiglitazone does not seem to be due to changes in mTORC2 activity. Finally, although rapamycin does not appear to modulate adiponectin production by adipose tissue (Refs. 6 and 16 and Table 2), a contribution of this adipokine to the rosiglitazone-mediated amelioration of hepatic glucose homeostasis cannot be excluded.

In addition to hepatic glucose production, we next investigated a possible involvement of adipose tissue and skeletal muscle glucose uptake in the improvement of glucose homeostasis induced by rosiglitazone in rapamycin-treated rats. Confirming and extending previous findings (9), rapamycin markedly reduced insulin-stimulated glucose uptake in both adipose tissue and skeletal muscle, with such tissues being responsible for the larger part of insulin-stimulated glucose disposal upon feeding. Surprisingly, rosiglitazone partially reverted the rapamycin-induced impairment of insulin-stimulated glucose uptake in skeletal muscle but not in adipose tissue, a tissue that,

Fig. 6. Protein content of phosphorylated (p)-Akt Ser473 and p-S6 Ser236 normalized for β-actin in liver (A–C) and gastrocnemius skeletal muscle (D–F) of rats treated with Rap or RSG for 15 days; n = 6 for each group. Means not sharing a common letter are significantly different from each other; P < 0.05.
due to its high levels of PPARγ expression, is one of the main targets of rosiglitazone. Noteworthy is that the rapamycin-induced impairment of insulin’s ability to stimulate glucose uptake in skeletal muscle and adipose tissue was associated with a significant reduction in p-Akt Ser473 content, as shown here and previously (6), indicating a possible involvement of mTORC2 inhibition in these specific rapamycin actions. Further supporting this hypothesis, in association with the reestablishment of insulin-stimulated glucose uptake, rosiglitazone restored the content of p-Akt Ser473 exclusively in skeletal muscle but not in adipose tissue (6).

The mechanisms underlying the tissue-specific action of rosiglitazone on mTORC2 activity and p-Akt Ser473 are unknown and clearly deserve further investigation. Previous studies have shown that rapamycin inhibits mTORC2 by forming a complex with FKBP12 that sequesters free mTOR, thereby reducing its availability for mTORC2 formation, a phenomenon that is cell type specific (34). Our findings confirm these in vitro observations and indicate that the diabetes-like syndrome induced by rapamycin with the dose and treatment regimen used here is a multifaceted condition that depends upon the tissue specificity of rapamycin action upon mTOR complexes. Indeed, whereas the increases in hepatic gluconeogenesis and β-cell insulin secretion brought by rapamycin seem to be due to mTORC1 inhibition only, the reduction in adipose tissue and skeletal muscle insulin-stimulated glucose uptake appears to be due to concomitant mTORC1 and -2 inhibition. Independently of affecting one or both complexes, rosiglitazone effectively attenuates the deleterious metabolic effects of rapamycin without impairing rapamycin antiproliferative and immunosuppressive actions, as estimated by a lymphocyte proliferation assay (data not shown), and despite having some of its actions antagonized by rapamycin. Indeed, we have shown here and previously (6) that rapamycin abrogates the reduction in visceral fat and plasma TAG levels and attenuates the increase in subcutaneous adiposity and adiponectin induced by rosiglitazone. This may explain partly why rosiglitazone does not completely revert the glucose intolerance induced by rapamycin.

In conclusion, our findings indicate that pharmacological PPARγ activation, eventually using ligands with minimal side effects, may represent a good strategy to attenuate the disturbances in glucose homeostasis typically seen upon rapamycin pharmacotherapy. The elucidation of the mechanisms by which rapamycin induces a diabetes-like syndrome and the development of strategies such as that proposed here to overcome its major deleterious metabolic consequences are of foremost importance to improve the therapeutic efficacy of rapamycin and analogs.

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GRANTS

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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise, relevant to this article.

AUTHOR CONTRIBUTIONS


REFERENCES


